Question #61233, Biology, Biochemistry

Antibiotics being used as a selective marker. (ampicillin and tetracycline)
After the steps taken to distinguish between successful and failed insertion with ampicillin, B-galactosidase is a secondary test. I'm struggling to understand this section before tetracycline is introduced in the media. Thank you!

Answer:

Described procedure refers to gene cloning.

In this process, a gene of interest is inserted into a simple quick-growing organism like *Escherichia coli* so that it produces a desired protein (eg: human insulin). This is done by neatly “cutting” a plasmid DNA at certain points using restriction endonucleases and then ligating the also-well-cut gene of interest with the plasmid, so that the two fit in like lock-and-key.

Figure 1 represents a simplified map of a typical plasmid:

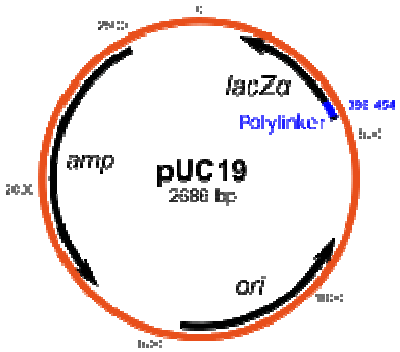

Figure 1 - Simplified map of a typical plasmid

However, current methods of transfection, i.e. the process of introducing this recombinant plasmid with the help of a vector (a carrier for the plasmid, such as lentivruses or liposomes) are quite inefficient. This means that not all the *E.coli* cells will be successfully transformed, so we need a method to detect the ones that have been transformed.

To selectively allow only transformed cells to grow, additional genes such as lacZ and ampR are also cloned into the recombinant plasmid. While ampR provides resistance to the antibiotic ampicillin, lacZ codes for a part of the enzyme b-galactosidase. If inserted into the right strain (variety) of *E.coli*, which already has the genes for the rest of the b-galactosidase protein chain, the entire enzyme can be produced only by all transformed bacteria.

So now we know that all the *E.coli* that have been successfully transformed also carry the ampR and lacZ genes, we can grow them on a medium containing ampicillin and X-gal. The latter ingredient is a substrate for the b-galactosidase enzyme and one of the products of their reaction is a blue colored substance. Thus, successfully transformed bacteria will produce blue colored colonies, and we can then isolate these cultures and mass-produce them to derive our protein of interest.

A successful round of gene cloning can make your plate (where the *E.coli* are grown) look like this. The white colonies have not been able to successfully produce b-galactosidase, but it seems like they do have ampR.